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In vitro studies on anti-leukemic effects of tetracycline's analogues and cyclophosphamide

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ABSTRACT

Leukemia is a heterogeneous group of hematological malignancies that originate from the bone marrow. Leukemias are classified as acute or chronic. Although acute leukemia affects patients of all ages, acute lymphoid leukemia is predominant in children and young adults while acute myeloid leukemia (AML) is predominant in the elderly. Combination chemotherapy results in complete remission in most of the patients; however, relapse occurs within 1-2 years. After relapse, more intensive treatment is usually unsuccessful and associated with high toxicity. Chronic myeloid leukemia (CML) is characterized by Bcr/Abl fusion tyrosine kinase that renders CML untreatable with conventional chemotherapy. Drugs specifically targeting the Bcr/Abl molecule have been developed, but acquired resistance in CML cells jeopardizes treatment success. Therefore, it is important to investigate new treatment approaches and strategies. Tetracyclines were discovered during the 1940's as antibiotics. Further development resulted in new analogues, some of them possessing essential non-antibiotic properties. Tetracycline analogues (TCNAs) were reported to inhibit cell growth in different tumor cell lines and cancer models. Their anticancer potency is attributed partially to inhibition of matrix metalloproteinases (MMP) and mostly to an apoptosis inducing effect. Cyclophosphamide (Cy) is an anticancer drug that is used in the treatment of several cancers including leukemia. The aim of the present thesis was to study TCNA-induced cytotoxicity and its underlying mechanisms in leukemic cell lines and to assess the mechanisms underlying cyclophosphamide cytotoxicity *in vitro* in the leukemic HL60 cell line.

The anti-leukemic effects of TCNAs doxycycline (Doxy), minocycline (Mino) and chemically modified tetracycline-3 (COL-3) were studied in the human leukemic cell lines AML HL60, lymphoblastic Jurkat, AML stem cell like KG1a and CML K562. TCNAs reduced the viability of leukemic cells in a concentration-dependent manner with decreasing sensitivity (HL60/Jurkat > K562 > KG1a). Further studies on molecular mechanisms of TCNAs-induced cell death were performed in HL60 and K562 cells. In HL60 cells, all three TCNAs induced apoptosis via mitochondria-mediated and caspase-dependent pathways. COL-3 exerted the strongest anti-proliferative and pro-apoptotic effect. In K562 cells, doxycycline and minocycline induced cell death that was dependent on caspase activity as well as mediated by nuclear DNA damage, increased lysosomal activity, Bcl-xL deamidation and activation of mitochondrial apoptotic pathways. However, COL-3 affected K562 cells by targeting the mitochondria and the nuclear DNA in a concentration-dependent manner. COL-3 in concentrations above IC₅₀ induced mitochondrial and DNA changes concomitantly, whereas COL-3 at lower concentrations primarily affected the mitochondria. The COL-3-induced cell death was independent of caspase activity, Bcl-xL and intact p53, but mediated by calpain and apoptosis inducing factor (AIF). Cy *in vitro* activation in HL60 cells was catalyzed by CYP2J2 as demonstrated by reduction of Cy metabolism and Cy-induced cytotoxicity by telmisartan, a selective inhibitor of CYP2J2 enzyme. The role of CYP2J2 in the Cy metabolism was further confirmed by incubation with recombinant CYP2J2 enzymes *in vitro*. Additionally, the CYP2J2 gene is highly expressed in patients with hematological malignancies.

In conclusion: TCNAs possess anti-leukemic activity and induce cell death stimulated by DNA damage. The cell death signals are exchanged between nucleus, mitochondria, lysosomes and ER. Caspases, calpain and AIF are core machinery for the cell death execution in this model. CYP2J2 is involved in bioactivation of Cy in leukemic cells. Studies on combination treatment with Cy and TCNAs are warranted.